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Bioremediation of Soil Polluted with Cadmium and Zinc Using Microflora from Abattoir Effluent

M. S. Mshelia*, A. N. Jones, S. G. Yadima

Department of Civil and Water Resources Engineering, University of Maiduguri, Maiduguri, Borno State, Nigeria.
*msmshelia01@unimaid.edu.ng

Research Article

Abstract

Heavy metals are toxic and detrimental environmental pollutants. Their release into the environment could be a major cause of water and soil pollution majorly exacerbated by industrialization. This study was conducted to assess the ability of abattoir effluent to remediate soil contaminated with heavy metals such as, cadmium and zinc. Soil sample was artificially contaminated by adding 50g of $Cd(NO_3)_2XH_2O$ and $ZnClXH_2O$ to uncontaminated soil. The bacteria isolated and characterized from the abattoir effluent were Salmonella sp., Escherichia coli, Staphylococcus sp., Shigella sp., Pseudomonas sp., Enterobacter sp., Proteus sp., and Micrococcus sp. while the fungi species analyzed includes Aspergillus niger, Penicillium sp, Trichoderma sp, and Rhizopus sp. in the abattoir effluent. After remediation, the results showed significant ($P < 0.05$) reductions in heavy metal concentrations in the soil samples. Specifically, sample containing 200ml of abattoir effluent had the highest cadmium removal efficiency of 98% while sample containing 300ml of abattoir effluent recorded 79% removal performance in zinc. The study showed that microflora in abattoir effluent have the potential of removing heavy metals such as cadmium and zinc from polluted soil over a period of 3 weeks.

Keywords
Abattoir Effluent; Bioremediation; Cadmium and Zinc pollutants; Microflora; Soil pollution

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1. Introduction

The global industrial revolution has resulted in the release of harmful substances into the environment, thus compromising the world's ecological equilibrium system. The release of pollutants such as heavy metals into the environment due to human activities has been a major cause of water and soil pollution. Interestingly, in most developing countries, industries tend to generate wastes that are frequently discarded in the environment which are detrimental to public health. Small and large-scale companies are the culprits in this regard (Fischer and Glaser, 2012; Ubwa *et al.,* 2013; Godambe and Fulekar, 2016; Mahopatra *et al.,* 2019). It is noteworthy that these are generally exacerbated by their buildup in the environment through the food chain (Afolayan, 2018). Several ailments affecting human beings in recent years are consequences of persistent exposure to pollution in the environment (Ire *et al.,* 2017; Li *et al.,* 2020; Goutam *et al.,* 2021). Heavy metals are naturally occurring components of the environment, but their improper usage has changed their geochemical cycles and biological equilibrium (Moses and Obasigie, 2020). Interestingly, heavy metals such as cadmium, copper, lead, nickel, zinc, arsenic etc. are being discharged into the environment which are toxic even at extremely low quantity (Dixit *et al.,* 2015; Huang and Keller, 2020). Currently, there are several methods being adopted in removal of heavy metals from a system e.g. chemical precipitation, ion exchange, adsorption, ultra-filtration, reverse osmosis, electro dialysis, coagulation, flocculation and floatation (Yadav *et al.,* 2017; Atikpo and Micheal, 2018; Faheem *et al.,* 2020;). However, traditional cleanup techniques are no longer cost-effective or beneficial, necessitating the development of a more cost-effective and efficient alternative to tackle the danger of pollution (Agamuthu *et al.,* 2013). It is noteworthy that bioremediation is a new and promising method for removing heavy metals from contaminated environment. These concerns have resulted to resurgence of interested in creating ways to clean up contaminated settings as the public becomes more aware of the consequences of polluted surroundings to human and animal health (Barakat *et al.,* 2016). The introduction of microorganisms and plants to remove, contain, or render a pollution harmless is known as bioremediation. It also entails the use of carefully chosen or genetically altered microbes and plants to absorb toxins directly from the environment. Both organic and inorganic pollutants can be remedied using bioremediation in both solid and liquid substrates (Truu *et al.,* 2015; Vyas and Waoo, 2020; Adetunji and Anthony, 2021). In comparison to other typical cleanup processes, bioremediation is a promising strategy that has been employed in nature and is cost-effective, ecologically friendly, efficient, and has socioeconomic value, as well as being environmentally sustainable (Mishra *et al.,* 2017; Lal *et al.,* 2018). Bioremediation can either be in-situ or ex-situ. In-situ bioremediation entails treating contaminated media on-site and is based on the destruction or transformation of pollutants, their

immobilization to lower bioavailability, and the separation of contaminants from the medium while ex-situ bioremediation necessitates the removal and transfer of contaminated material to a containment facility for treatment (Ghosh and Singh, 2005; Azubuike *et al.,* 2016; Vyas and Waoo, 2020). Strategies used in bioremediation are; biostimulation (stimulating viable native microbial population), bioaugmentation (introduction of artificial viable population), bioaccumulation (use of live cells), biosorption (use of dead microbial biomass), phytoremediation (use of plants) and rhizoremediation (use of plant and microbe interaction) (Da Silva and Alvarez, 2010; Shilpi, 2012).Microorganisms play a significant role in the rehabilitation of heavy metal-contaminated environments because they can withstand the toxicity of heavy metals in a variety of ways. Numerous heavy metals are separated, precipitated, and their oxidation states exceedingly changed by microorganisms (Gupta *et al.,* 2016; Alori *et al.,* 2018; Atigh *et al.,* 2020). Conversely, rather than employing a single bacterium strain, bioremediation will be successful if various bacteria strains are employed in the process (Kang *et al.,* 2016; Ojuederie and Babalola, 2017; Kure *et al.,* 2018). Additionally, several factors, such as water, oxygen, and the use of nitrogen and phosphorus supplies must be considered before a competent microbe breaks down a contaminated carbon source. The remediation process will be rendered ineffective if any of the given criteria are skipped. Furthermore, seeded cultures, biostimulation, bioaugmentation, and nutrients can all be used to improve the efficacy of pollution breakdown (Tyagi *et al.,* 2011). The fundamental goal of bioremediation is to enhance the ability of the local microflora in a contaminated environment to create more enzymes that will supply more food to improve their growth (Pratush *et al*., 2018).

Abattoir effluent is thus, believe to contain several microbes, emanating from animal intestine which could be vitally used in bioremediation. Additionally, these are an essential part of cattle business in Nigeria. Over 150 million Nigerians rely on the sector for their domestic meat supply while other Nigerians work directly in the business to earn a living (Nafaranda *et al.,* 2005; Joseph *et al.,* 2021). Abattoirs are often located near streams and rivers, where untreated wastewater is released into water bodies (Sangodoyin and Agbawhe, 1992). In growing and heavily populated countries like Nigeria, contamination of natural water sources has been a major concern to the general public (Adesina *et al.,* 2018). Abattoir effluent contains substantial amount of biodegradable organic matter Ogbomida *et al.,* (2016) as well as high concentration of microbial population capable of removing pollutants Atuanya *et al.,* (2018) including microorganisms such as *Pseudomonas* sp, *Bacillus* sp, *Aspergillus* sp, *Trichoderma viride, Penicillium notatum,* etc (Ariyo and Obire, 2016; Ediene and Iren, 2017; Adegbite *et al.,* 2018). Therefore, this study aimed at evaluating the potential of using microflora from abattoir effluent to biostimulate, bioaugment and remediate soil contaminated with Cd and Zn.

2. Materials and Methods

2.1 Materials

The materials used in this research work includes; soil sample, abattoir effluent, distilled water. Laboratory grade cadmium and zinc as Cd $(NO_3)_2$. XH_2O and ZnCl, XH2O were also used in the study. Similarly, some of the equipment used in the study includes Microwave Digester Model: Master 40 by Sineo chemistry Technology, China, Atomic Absorption Spectrophotometer model:210VGP AAS by Buck scientific, USA, Muffle Furnace Model: FM 515 by p-select, Pizzato Italy, 15L Autoclave Sterilizer.

2.2 Methods

2.2.1 Sample collection and processing

Abattoir effluent was collected using a sterile 5L plastic gallon from Maiduguri central abattoir at the point of discharge in the slaughter house located in Gomboru ward in Maiduguri metropolis, Borno state Nigeria (11.858606^{o}N, 13.178652^{o}E). Topsoil was collected at a depth of 7-15cm behind the Environmental Engineering Laboratory, Faculty of Engineering, University of Maiduguri's (11.813049^{o}N, 13.205356^{o}E). To attain a homogeneous particle size, the soil sample was air-dried for 24 hours at room temperature in the laboratory before sieving using a 2mm mesh. The soil sample was again air-dried for 24 hours after being synthetically contaminated with 50g of Cd and Zn in the form of $Cd(NO_3)_2$ XH_2O and $ZnClXH_2O$ salts manufactured by Park Scientific, Northampton, England. The soil sample was placed in a plastic container with a diameter of 200mm and a depth of 150mm and labeled A, B, and C. Before adding the abattoir effluent, 60g of soil sample was collected from each container for heavy metal analysis to determine the Cd and Zn levels. Following that, sample A (Control sample) without abattoir effluent while 200ml of abattoir effluent was measured and added to Sample B, and 300ml of abattoir effluent was added to Sample C. Thereafter, all the samples in the containers were kept in the laboratory at room temperature for three weeks. Thereafter, all the samples were aerated and irrigated with a predetermine volume of distilled water i.e., 200ml at every 5-day interval. During the investigation, soil samples were taken from the samples every 7 days for heavy metal analysis. All analysis was conducted in triplicates.

2.3 Elemental Analysis with Atomic Absorption Spectrophotometer (AAS)

Heavy metal concentrations in the samples were measured using an Atomic Absorption Spectrophotometer (AAS 210 VGP, Buck Scientific Company, USA) at Yobe State University Department of Chemistry Analytical Laboratory, Yobe State. The absorbance of the heavy metals present in the soil sample was used to calculate their concentrations. The heavy metals present were determined using absorption/concentration mode, and the instrument output for each solution was recorded. The same analytical technique was used to determine heavy metal levels in digested blank solutions and spiked samples.

2.4 Microbiological analyses

Microbiological analyses were conducted at the Department of Biological Sciences laboratory, University of Maiduguri,

Borno State, Nigeria. To isolate bacteria from the effluent sample, 1 ml of effluent was diluted in 9 ml of distilled water. The combination was shaken vigorously and the resultant suspension was serially diluted in ten folds. Aliquots (0.1 ml) of each of the test tubes (10^{-2}, 10^{-3}, 10^{-5}) were distributed under aseptic conditions using the spread plate technique onto petri dishes containing nutrient agar, eosin methylene blue agar, mannitol salt agar and Mac Conkey agar that were prepared according the manufactures instructions and incubated at 37ºC for 24 hours. To isolate fungi from the effluent sample, the same procedure as described previously was used, using Sabouroud dextrose agar and cultured at 30ºC for 3 days. Following incubation, established colonies were selected based on their colony morphology and sub-cultured on nutrient agar for bacteria and Sabouroud dextrose agar for fungi to acquire pure samples before identifying the bacteria and fungi isolated. Furthermore, catalase test, methyl red, indole, oxidase, Voges-Proskaur, citrate, aerobic and anaerobic tests were performed on the bacterial isolates to identify them. Bacterial isolates were identified and described based on their biochemical properties, as well as Bergey's Manual of Determinative Bacteriology's scheme (Holt *et al*., 1994). The fungi was also identified based on their macroscopic and microscopic characteristics (Alkhalidi *et al.,* 2019) as well as the use of Smith's Introduction to Industrial Mycology (Onions *et al.,* 1981) and Introduction to Food-Borne Fungi (Samson and Reenen-Hockstra, 1988) as a guide.

2.5 Determination of total bacterial and fungal counts.

Abattoir effluent samples were diluted tenfold up to (10^{-5}) in sterile distilled water to enumerate the bacteria and fungi present in triplicates. Bacterial plate counts were performed using nutrient agar while Sabouroud dextrose agar was used for fungal counts all of which was done using the pour plate technique. This approach used successive dilutions of 0.1ml abattoir effluent samples that were pipetted into each sterile Petri-dish containing freshly prepared nutrient agar and Sabouroud dextrose agar for bacteria and fungi culture respectively, and incubated at 37°C. The total bacterial and fungal counts were determined using a colony counter and expressed as colony forming unit (CFU/ml).

$$\mathrm{CFU} = \frac{Number\ of\ colonies \times Total\ Dilution\ Factor\ (TDF)}{Volume\ of\ culture\ plated\ (ml)}$$

For bacteria, colonies between 30 and 300 were counted while fewer than 30 colonies and more than 300 colonies were considered too few to count (TFTC) and too numerous to be counted (TNTC) following Kurna *et al.,* (2016) while fungal colonies between 10 and 150 were counted while fewer than 10 and above 150 were neglected (ESA, 2012).

2.6 Data Analysis.

Data obtained in this work were subjected to Two-Way Repeated Measures using analysis of variance (ANOVA) to compare the decrease in heavy metal concentration in each sample tested and the level of significance adopted at 5%.

3. Results and Discussion

3.1 Bacterial and fungal isolates

Bacteria isolated from the abattoir effluent were identified as *Pseudomonas* sp., *Enterobacter* sp., *Staphylococcus* sp., *Salmonella* sp., *Shigella* sp., *E-coli* sp., *Proteus* sp., and *Micrococcus* sp. Similarly, fungi, *Aspergillus niger*, *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. were also identified in the abattoir effluent as shown in Table 1. Some of these microbes such as *Escherichia* sp., *Staphylococcus* sp., *Enterobacter* sp., *Salmonella* sp, *Aspergillus niger and Penicillium* sp are pathogenic (Kim *et al.,* 2018; Bano *et al.*, 2020). Pathogenic bacteria with similar characteristics have been identified from abattoir effluent in several studies (Rabah *et al.,* 2008; Ogunnusi and Dahunsi, 2014). Similarly studies were conducted to isolate *E.* coli, *Staphylococcus* sp, *Aspergillus* niger, *Enterobacter* sp. and *Penicillium* sp from abattoir effluent (Adesemoye *et al.,* 2006; Neboh *et al.,* 2013; Ogbomida *et al.,* 2016; Odeyemi *et al.,* 2017) all these studies are in tandem with the microbes isolated and identified from this study. The abundance and diversity of bacterial species detected in the abattoir effluent may be connected to the various stages of decomposition of the elements of abattoir effluent, which include blood, urine, body parts, dung, undigested grains, and grasses. While the prevalence of distinct fungal isolate communities in the abattoir effluent might be connected to the availability of substrates that the fungi can easily exploit for food and energy, as well as the effluent's favorable pH.

The colony morphology of bacteria isolated from abattoir effluent was studied. Form, texture, color, margin, elevation, and opacity were observed as characteristics (Table 1).

A total of eight bacterial isolates were discovered in the effluent, and the colonies of the bacterial isolates were primarily circular, with a single contoured form being seen. The bacterial isolates' surfaces were discovered to be smooth and shiny. Colonies from the isolates were white, creamy, reddish/pinkish, greenish, and yellow in color. The bacterial isolate colonies' margins were discovered to be entire and lobate. The majority of the bacterial isolates had convex, flat, and raised elevation with opaque or transparent opacity. Six of the eight bacteria isolates were Gram negative and rod shaped, while the other two were Gram positive and cocci. The morphological characteristics of identified fungi isolates in abattoir effluent sample is shown in Table 2. *Aspergillus* niger colony morphology displays a black colony on top and grey colonies on the reverse, and the microscopic view illustrates the arrangement of conidia.

Table 1: Morphological and biochemical observations of different bacteria isolated from abattoir effluent sample.

Form	Circular	Circular	Contoured	Circular	Circular	Circular	Circular	Circular
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Shiny	Smooth
Colour	Yellow	White	Cream	Yellowish	Colorless	Greenish	White	Reddish-Pinkish
Margin	Entire	Entire	Lobate	Entire	Entire	Entire	Entire	Lobate
Elevation	Flat	Convex	Flat	Raised	Convex	Convex	Convex	Slightly raised
Opacity	Transparent	Transparent	Transparent	Opaque	Transparent	Rough	Moist	Opaque
Shape	rod	rod	rod	cocci	rod	rod	rod	cocci
Gram Reaction	-	-	-	+	-	-	-	+
Catalase	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	+	-	+
Citrate	+	-	+	-	-	+	+	-
MR	+	+	+	+	+	+	-	-
VP	-	-	+	-	-	-	+	-
Indole	+	+	-	-	+	-	-	-
Bacteria Identified	*Salmonella sp.*	*Escherichia-coli.*	*Proteus sp.*	*Staphylococcus sp.*	*Shigella sp.*	*Pseudomonas sp.*	*Enterobacter sp.*	*Micrococcus sp.*

Table 2: Morphological characteristics of identified fungi in abattoir effluent sample

S/No	Macroscopic Features	Microscopic Features	Colony Color Reverse	Fungi Isolates
1	Filamentous mold, medium in size, has a dark margin and turns black after 72hrs.	Has a double-walled conidiophore that was hyaline and smooth with warty nature.	Grey	*Aspergillus niger*
2	Rapid colony growth, greenish-white shade, filamentous, velvety.	Has dense felt of conidiophore, conidiophore can be single or doubled, has a hyaline conidiophore and smooth or rough walled.	Pale-Yellowish	*Penicillium sp.*
3	Rapid colony growth, cotton candy like, white with grey-black top that darkens overtime.	Has unbranched sporangiophores that are long, rhizoids were seen on its sporangiophores.	White	*Rhizopus sp.*
4	Colony white mycelium with dark green conidia	Conidiophores have long repeated branches, with shorter branches near the apex, it has a smooth-walled conidia.	Colorless	*Trichoderma sp.*

Penicillium sp possesses morphological characteristics such as a greenish-white top surface, pale yellow on the reverse, and has a hyaline conidiophore with smooth or rough wall. *Rhizopus* sp has a profound cotton texture with a white to gray-black tint on top and a pale white color on the reverse, as well as a black pigment sporangium. *Trichoderma* sp has a morphological feature with white mycelium and dark green conidia surface on the top, colorless on the reverse, and microscopically has a Conidiophores with long repeated branches, shorter branches near the apex and smooth-walled conidia.

3.2 Enumeration of bacteria and fungi in abattoir effluent. Table 3 shows the microbial counts in abattoir effluent. The results show that the bacterial count ranged from $2.45x10^4$ - $6.04x10^8$ CFU/ml while the count associated with fungal count ranged $4.54x10^2$ - $4.82x10^4$ CFU/ml respectively. In addition, *Salmonella* sp and *Micrococcus* sp have the highest count of $6.04x10^8$ CFU/ml while Pseudomonas sp. and Staphylococcus sp. has the least count of $2.45x10^4$ and $7.35x10^4$ CFU/ml respectively, *Rhizopus* sp has the highest count of $4.82x10^4$ CFU/ml while *Aspergillus niger* has the lowest count of $4.54x10^2$ CFU/ml as indicated in Table 3.

Table 3: Mean Bacterial and Fungal count for abattoir effluent.

S/No.	Bacteria	Counts (CFU/ml)	FUNGI	Counts (CFU/ml)
1	*Salmonella sp.*	$6.04x10^8$	*Aspergillus niger*	$4.54x10^2$

2	*Escherichia-coli.*	$3.11x10^7$	*Penicillium sp.*	$3.00x10^4$
3	*Proteus sp.*	$2.62x10^7$	*Rhizopus sp.*	$4.82x10^4$
4	*Staphylococcus sp.*	$7.35x10^4$	*Trichoderma sp.*	$1.12x10^3$
5	*Shigella sp.*	$3.65x10^6$		
6	*Pseudomonas sp.*	$2.45x10^4$		
7	*Enterobacter sp.*	$1.85x10^6$		
8	*Micrococcus sp.*	$6.04x10^8$		

***CFU/ml;** Colony Forming Unit/Milliliter.

3.3 Cadmium (Cd).

The initial Cd concentration in sample A (Control) was 5.270.32 mg/kg, which decreased to 4.74±0.26 mg/kg (Table 4) without treatment. Similarly, before remediation with abattoir effluent, the initial concentration in sample B (200ml Abattoir Effluent) was 5.27±0.36mg/kg. When compared to the initial concentration, the concentration reduced during the first, second and the third week respectively with Mean±SD values of 3.38±0.27mg/kg, 2.44±0.22mg/kg, and 0.08±0.11mg/kg. The decrease in Cd content in the sample might be connected to a study by Henao and Ghneim-Herrera, (2021) which suggests *Enterobacter* sp. can be used to remediate cadmium-polluted soil owing to the bacteria's intracellular cadmium accumulation (Ghosh *et al.*, 2022).

Table 4: The mean standard deviation concentration for Cadmium in soil remediated with Abattoir effluent

Sample Id	**Initial Conc.**	**Week 1**	**Week 2**	**Week 3**
	$\bar{X}$±SD	**$\bar{X}$±SD**	**$\bar{X}$±SD**	**$\bar{X}$±SD**
A - Control	5.27±0.32	4.88±0.23	4.74±0.26	4.63±0.16
B – 200ml AE	5.27±0.36[a]	3.38±0.27[b]	2.44±0.22[c]	0.08±0.11[d]
C – 300ml AE	5.89±1.09[a]	3.84±0.80[b]	2.82±0.66[c]	0.33±0.28[d]

*** AE** Abattoir Effluent

*Values with different superscripts within a row are significantly different ($P < 0.05$).

The concentration of Cd in sample C (300ml Abattoir Effluent) was 5.89±1.09mg/kg prior to remediation. However, after remediation, the concentration significantly ($p<0.05$) decreased to 3.84±0.80mg/kg, 2.82±0.66mg/kg, and 0.33±0.28mg/kg after week 1, 2 and 3 respectively. The reduction in Cd content was in agreement with a study conducted by Vullo *et al.*, (2008) that indicates *Pseudomonas* sp, may be used as a suitable biosorbent for the removal of Cd in polluted soil. The initial Cd concentrations in all of the samples were higher than the WHO, USEPA, and NESREA stipulated levels (0.3 mg/kg, 0.48 mg/kg, and 3 mg/kg, respectively). The concentration of Cd in the samples remained over the USEPA, NESREA, WHO standards after the first week, however, during the second week, the Cd concentration in sample B and C decreased to a value below the 3mg/kg NESREA limit but above the WHO and USEPA limits. However, the Cd concentration in sample B and C declined well below the USEPA, NESREA, WHO acceptable limits for Cd in soil by the third week after remediation (WHO, 2008; NESREA, 2009).

The percentage decrease in the concentration of Cd in the soil samples remediated with abattoir effluent for three weeks showed a gradual decrease in the concentration of Cd in the control sample (A) from 7% in the first week to 10% in the second week and 12% in the third week (Fig. 1).

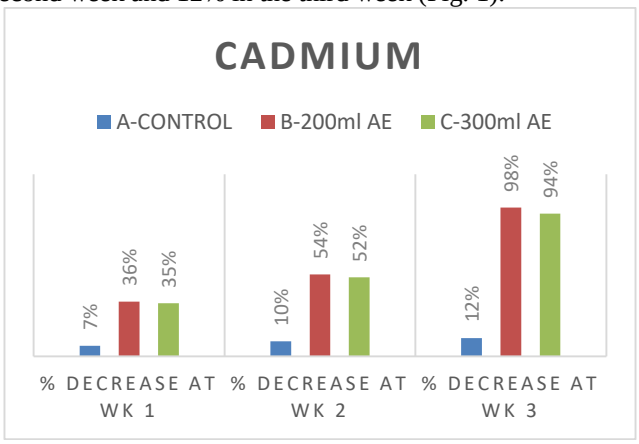

Figure 1: Percentage decrease in Cd concentration at different weeks.

However, comparing the percentage reduction of cadmium in samples B and C remediated with abattoir effluent indicated that the decrease is in a higher percentage ranging from 36% - 98% and 35% - 94% for sample B and C respectively from the first week to the third week after the remediation process. Sample C has the lowest percentage decrease of 35% while sample B has a percentage decrease of 36% in the first week. In the second week sample C has a percentage decrease of 52% compared to sample B which has a decrease of 54%. In the third week, sample C has a 94% decrease in cadmium concentration while sample B has the highest percentage decrease of 98% after remediation with the effluent.

3.4 ZINC (Zn).

The initial concentration of Zn in sample A (control) was 44.18±0.21mg/kg. After three weeks, the concentration of Zn in the control sample was noted as 39.67±0.30mg/kg, 36.31±0.34mg/kg, and 34.53±0.51mg/kg for the first, second, and third weeks respectively (Table 6). In sample B (200ml abattoir effluent) a significant decrease was noted in the initial

concentration of Zn in the sample compared to the Zn concentration in the first, second and third weeks after remediation with abattoir effluent (48.37±1.80mg/kg, 27.81±1.77mg/kg, 21.22±2.28mg/kg and 10.64±1.01mg/kg). The decrease in Zn concentration in the sample may be traced to studies by Ahemad and Malik, (2012) and Nwagwu *et al.*, (2017) that *Pseudomonas* sp. and *Escherichia-coli* can be used to remediate soil polluted with zinc due to the active biosorption and bioaccumulation capabilities of *Pseudomonas* sp. and *Escherichia-coli* via the sorption mechanism.

Table 6: The mean concentration for Zinc in soil remediated with Abattoir Effluent at different weeks.

SAMPLE ID	WEEK 0	WEEK 1	WEEK 2	WEEK 3
	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SD
A - CONTROL	44.17±0.21	39.67±0.30	36.31±0.34	34.53±0.5
B – 200ml AE	$48.37±1.80^{a}$	$27.81±1.77^{b,a}$	$21.22±2.28^{c,b,d}$	$10.64±1.01^{d}$
C – 300ml AE	$51.46±5.43^{a}$	$28.91±3.69^{b,a}$	$21.27±3.12^{c,b,d}$	$10.87±1.04^{d}$

*** AE** Abattoir Effluent,

*Values with different superscripts within a row are significantly different (P < 0.05).

Before remediation, the Mean±SD concentration of Zn in sample C (300ml Abattoir Effluent) was 51.46±5.43mg/kg. Following remediation, the concentration significantly decreased in the first, second, and third weeks (28.90±3.69mg/kg, 21.27±3.12mg/kg and 10.87±1.04mg/kg) respectively. The reason for the decline in Zn level in the sample can be attributed to the presence of *Bacillus* sp and *Staphylococcus* sp in the abattoir effluent that was used in spiking the soil sample for the remediation as stated by Nwagwu *et al.*, (2017), and El-Barbary and Hafez, (2018) in their studies. The concentration of Zn in the samples were all below the USEPA, NESREA, and WHO permissible limits of 1100 mg/kg, 421 mg/kg, and 50 mg/kg. However, following remediation with the abattoir effluent, the concentration of Zn in sample B and C decreased significantly compared to the control sample (A).

The percentage reduction in zinc content in soil samples remediated with abattoir effluent for three weeks revealed that the zinc content in the control sample (A) has decreased from 10% in week 1 to 18% in week 2 and 22% in week 3. When the percentage reduction of zinc in samples B and C spiked with abattoir effluent as compared to the control sample, the decrease is greater, ranging from 43% to 78% (Fig. 2).

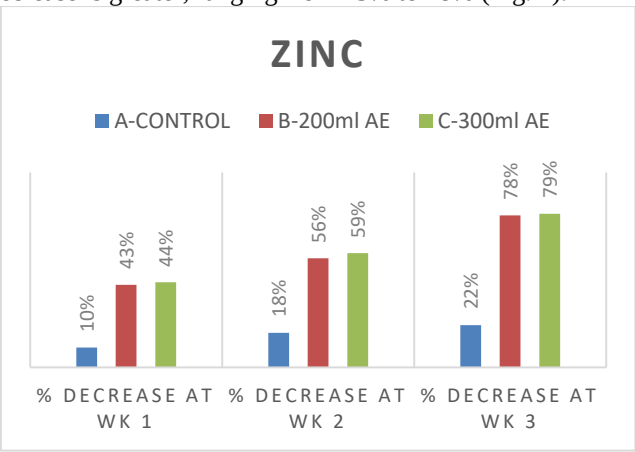

Figure 2: Percentage decrease in zinc concentration at different weeks.

Sample B had the lowest percentage reduction of 43% in the first week, while sample C has the greatest percentage loss of 44%. In week 2, sample B continues to have a lower percentage reduction of 56%, but sample C has a 59% decrease. Sample B had a 78% reduction in zinc levels after remediation with the effluent, whereas sample C has the greatest percentage decrease of 79%.

4. Conclusion

This study assessed the potential of abattoir effluent in bioremediating soil polluted with heavy metals (Cd and Zn). The study has shown that abattoir effluent possesses diverse microbes that can absorb and bioaccumulated heavy metals especially Cd and Zn either intracellularly or extracellularly. The Cd and Zn polluted soil were successfully remediated using abattoir effluent as a stimulant for the remediation achieving efficiencies of 98% for Cd and 79% for Zn. Even though, the concentration of Cd and Zn in the soil samples exceeds the WHO, USEPA and NESREA stipulated limits, the concentration reduced significantly following treatment using abattoir effluent. The findings of the study make it evident that abattoir effluent has the potential to be used in the process of remediating soil that is polluted with heavy metal Cd and Zn.

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